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Measuring Endogenous Phytohormones

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An abbreviated version of this protocol was published in Plant Physiology in Jun 2020

Rice GROWTH-REGULATING FACTOR7 Modulates Plant Architecture through Regulating GA and Indole-3-Acetic Acid Metabolism1, [OPEN]

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Detailed protocol

Equipment

1. UPLC (Ultra Performance Liquid Chromatography), Shim-pack UFLC SHIMADZU CBM20A, http://www.shimadzu.com.cn
2. MS/MS (Tandem mass spectrometry), Applied Biosystems 4500 QTRAP, http://www. appliedbiosystems.com.cn
3. WatersACQUITY UPLC HSS T3 C18, 1.8 μm, 2.1 mm * 100 mm

Recipes

1. mobile phase: ultrapure water with 0.1 % methanoic acid
2. organic phase: acetonitrile with 0.1 % methanoic acid

Detailed protocol

1. 15 seeds of the *OsGRF7* transgenic plants were planted in a container filled with soil with 2- × 2-cm spacing in a phytotron.
2. Fresh 15-d-old seedlings were harvested, weighed, and then immediately ground into powder in liquid nitrogen. Since tissue close to the soil may be contaminated, approximately 0.5 cm of tissue near the soil was removed with scissors.
3. 100 mg powder was weighed for subsequent phytohormone extraction.
4. After being extracted with 1mL of 80 % (v/v) methanol at 4 °C for 12 h, the extract was centrifuged at 12,000 rpm under 4 °C for 15 min.
5. The supernatant was collected and evaporated to dryness under a nitrogen gas stream at 35 °C , and then reconstituted in 100 mL of 95% (v/v) acetonitrile.
6. Then supernatant was collected for liquid chromatography-mass spectrometry analysis after the solution was centrifuged at 12,000 rpm under 4 °C for 15 min.
7. Quality control (QC) samples are prepared from a mixture of sample extracts for analysis of samples under the same treatment method. To analysis the repeatability of the analysis procedure, in the course of instrumental analysis, one quality control sample was inserted for every 10 test samples.
8. The sample extracts were analyzed using an LC-ESI-MS/MS system. The analytical conditions were as follows, HPLC: column, Waters ACQUITY UPL HSS T3 C18 (1.8 μm, 2.1 mm*100 mm); solvent system, water (0.1% acetic acid): acetonitrile (0.1% acetic acid); gradient program, 100:0 V/V at 0 min, 5:95 V/V at 11.0 min, 5:95 V/V at 12.0 min, 95:5 V/V at 12.1 min, 95:5 V/V at 15.0 min; flow rate, 0.40 ml/min; temperature, 40 °C; injection volume: 5 μl. The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap (Q TRAP)-MS.
9. LIT and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (Q TRAP), API 4500 Q TRAP LC/MS/M System, equipped with an ESI Turbo Ion-Spray interface, operating in a positive ion mode and controlled by Analyst 1.6 software (AB Sciex). The ESI source operation parameters were as follows: ion source, turbo spray; source temperature, 550 °C; ion spray voltage (IS), 5500 V; ion source gas I (GSI), gas II (GSII), curtain gas (CUR) were set at 55, 60, and 25.0 psi, respectively; the collision gas (CAD) was high. Instrument tuning and mass calibration were performed with 10 and 100 μmol/L polypropylene glycol solutions in QQQ and LIT modes, respectively. QQQ scans were acquired as MRM experiments with collision gas (nitrogen) set to 5 psi. DP and CE for individual MRM transitions were done with further DP and CE optimization. A specific set of MRM transitions were monitored for each period according to the plant hormones eluted within this period.
10. Qualitative analysis was made on the first and second spectrum data of mass spectrometric detection based on the self-built database and the public database of metabolite information

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Chen, Y. , Dan, Z. and Li, s. (2021). Measuring Endogenous Phytohormones. Bio-protocol Preprint. bio-protocol.org/prep1200.
2. Chen, Y., Dan, Z., Gao, F., Chen, P., Fan, F. and Li, S.(2020). Rice GROWTH-REGULATING FACTOR7 Modulates Plant Architecture through Regulating GA and Indole-3-Acetic Acid Metabolism1,[OPEN]. Plant Physiology 184(1). DOI: 10.1104/pp.20.00302